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(57) Abstract

Method for treatment of a patient having a normal level of activity of a naturally occurring protein, or having a condition characterized by the presence of an elevated or reduced level of that natural protein. The method includes administering to the patient nucleic acid which encodes an agonist or inhibitory polypeptide of the biological activity of the natural protein, or of a naturally occurring effector which affects the biological activity of that natural protein. The agonist or inhibitor is a polypeptide (i.e. an amino acid chain including at least 2 amino acids, and generally up to about 500 amino acids) expressed from the nucleic acid when that nucleic acid is present in the patient. This agonist and inhibitory polypeptide act *in vivo* to increase or reduce respectively the specific biological activity of the natural protein or the naturally occurring effector in the patient.

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IN VIVO PEPTIDE THERAPY

Cross Reference to Related Applications

This application is a continuation-in-part of Serial No. 858,128, filed March 26, 1992; the disclosure of this application is incorporated herein by reference.

Field of the Invention

This invention relates to gene therapy for treatment of diseases not caused by genetic deficiencies.

Background of the Invention

Dick, et al. Trends in Genetics June 1986, p. 165, describes the use of retrovirus vectors for introducing genes into cells. They state that this technology allows consideration of the feasibility of introducing appropriate genes into human bone marrow with the aim of correcting certain genetic defects. They further state that

> [a]lthough several thousand genetic diseases have been identified in man, only those which affect the hematopoietic system and can be corrected by gene transfer into bone marrow stem cells can be considered as candidates for gene therapy at present. Some genes (e.g., globin) will need to be

> expressed in the appropriate cells at precise levels in order to have a therapeutic effect and to avoid killing the cell. Initially, the most suitable diseases for gene therapy are life threatening diseases where the levels and lineage specificity of expression need not be stringently regulated. One such candidate is adenosine deaminase deficiency disease which leads to a severe combined immunodeficiency (SCID).

Id. at 169-170. Anderson, 216 Science 401, 1984 describes gene therapy as insertion of a normal gene into an organism to correct a genetic defect. It describes various techniques available for such therapy in humans and other organisms. Costantani, et al., 233 Science 1192, 1986 described correction of murine ß-thalassemia by gene transfer to a germ line as an example of gene therapy, and as a preliminary step toward gene therapy in humans.

More recently, a gene therapy method was described for treatment of hypercholesterolemia. Angier, New York Times Medical Science, October 29, 1991 at B6 describes use of "true gene therapy" where a gene encoding a lipoprotein receptor protein is provided to patients lacking such a gene.

BioVenture View (Feb. 1992, not admitted to be prior art to the present invention) describes a method for insertion of foreign genes into fibroblasts without use of viral vectors. They suggest delivery of insulin, LDL receptors and calcitonin.

SUMMARY OF THE INVENTION

This invention concerns in vivo expression of polypeptides active as agonists or as inhibitors of protein activity. The activity inhibited includes that of proteinases, anti-proteinases, and other enzymes or hormones as well as the activity of receptors for hormones or other endogeneous molecules, into a living animal by introduction of nucleic acid encoding such an agonist or inhibitor. Applicant has recognized that there are a variety of protein activities involved in various pathologic states. Such pathologic states occur in individuals without genetic defects. That is, the state is not caused by a severe lack of a protein activity within an individual caused by lack of naturally occurring nucleic acid encoding that activity (i.e., nucleic acid present in normal individuals within a population). Rather, the pathological state reflects an aberrant level of protein activity caused by some other mechanism or by more subtle regulations of protein activity or that the pathological state can be manipulated in spite of normal protein activity. Applicant's invention is based upon the therapeutic benefit that is achieved by delivering a gene or other nucleic acid that encodes an agonist or inhibitor of such a protein activity.

From the examples provided below, it will be clear that the method of this invention is distinct from gene therapy described in the Background of the Invention. This invention does not include treatment of patients suffering from diseases caused by genetic

deficiencies, in which a defective gene is "mended" by provision of a normal gene. Rather, a gene is generally introduced into a patient to modulate activity of a natural protein, or to supplement the normal activity of a protein.

DETAILED DESCRIPTION OF THE INVENTION

Thus, in a first aspect, the invention features a method for treatment of a patient having a normal level of activity of a naturally occurring protein, or having a condition characterized by the presence of an elevated or reduced level of that natural protein. The method includes administering to the patient nucleic acid which encodes an agonist or inhibitory polypeptide which mimics or inhibits the biological activity of the natural protein or the naturally occurring effector of that natural protein. The agonist or inhibitor is a polypeptide (i.e., an amino acid chain including at least 2 amino acids, and generally up to about 500 amino acids) expressed from the nucleic acid when that nucleic acid is present in the patient. This agonist or inhibitory polypeptide acts in vivo to reduce the specific biological activity of the natural protein or the naturally occurring effector in the patient. The agonists may also act as enhancers of protein activity.

The phrase "naturally occurring protein" is meant to include proteins produced by the majority of the group of animals to which the patient belongs, and includes those proteins produced at abnormal levels in an individual to be treated, but not those

proteins which are produced at abnormal levels because of a genetic defect in the structural gene encoding such a protein in the individual to be treated. Examples of such proteins include various cell receptors, cytokines, hormones, proteases and other enzymes. They do not include those proteins introduced by extraneous organisms such as viruses, bacteria or other infectious organisms.

In general, the term "polypeptide" is defined as a polypeptide which acts as an agonist, i.e., have an activity similar to the naturally occurring protein (examples of such polypeptides include erythropoietin, and follicular stimulating hormone), or as an antagonist, i.e., having an activity which blocks the activity of the naturally occurring protein (e.g., by blocking angiotensin II binding). Other polypeptides may inhibit enzymatic formation of a deleterious effector compound (e.g., inhibitors of the formation of renin, angiotensin I, or angiotensin II), may inhibit deleterious protein degradation (e.g., degradation by stromelysin, or collagenase), or may inhibit enzymatic degradation of beneficial effector compounds (e.g., neutral endopeptidase 24.11 degradation of atrial natriuretic factor, ANF, or angiotensin converting enzyme (ACE) degradation of bradykinin).

More specifically, the invention features inhibitory polypeptides active against various proteases, including: (a) inhibition of proteases that lead to disease, inhibition of proteases involved in the biosynthesis of peptide hormones (including inhibition of proteases involved in hypertension, e.g., the production

of renin, angiotensin I, angiotensin II, and endothelin, such as inhibition of proteases including prorenin protease, renin, ACE, and endothelin converting enzyme (ECE)); (b) inhibition of proteases directly involved in the disease process, including inhibition of proteases involved in inflammation processes, inhibition of proteases such as stromelysin, collagenases and other metalloproteases, inhibition of serine proteases involved in emphysema or arthritis (e.g., elastase), inhibition of proteases involved in tumor metastasis, and inhibition of proteases involved in tissue remodeling; (c) inhibition of proteases involved in the degradation of beneficial peptides, including inhibition of neutral endopeptidase to elevate ANF levels, and inhibition of ACE to elevate bradykinin levels. The invention also features polypeptides that antagonize peptide binding receptors and thereby produce a beneficial effect, including antagonizing the C-receptor of ANF to produce higher levels of ANF, antagonizing the angiotensin II receptor, and antagonizing the vasoactive intestinal peptide (VIP) receptor.

In preferred embodiments, the presence of the normal, elevated, or reduced level of the natural protein is a causative element in diseases such as hypertension (high blood pressure), cancer, inflammation, congestive heart failure, Alzheimer's disease and arthritis. In a most preferred embodiment, a protease involved in control of blood pressure is inhibited, e.g., angiotensin

converting enzyme, renin, prorenin protease, neutral endopeptidase 24.11 (ANFase or enklephalinase), or endothelin converting enzyme.

The inhibitory polypeptide may reduce the specific biological activity by direct interaction with the natural protein (e.g., by binding to the protein and thus inhibiting it in a competitive or non-competitive fashion). Alternatively, the agonist or inhibitory polypeptide may increase or decrease the specific biological activity of the natural protein by interacting with a naturally occurring effector compound and thus inhibiting the effect that such an effector has upon the activity of the natural protein or to the active site of the protein itself. For example, the natural protein may be a protease, and the effector an anti-protease. Inhibition of the anti-protease effector will increase the specific activity of the protease. An example includes the increased activity of atrial natriuretic factor upon inhibition of neutral endopeptidase 24.11 (ANFase or enklephalinase).

The inhibitory polypeptide may also reduce the specific biological activity (or increase such activity) by other competitive mechanisms, for example, by binding to a receptor for the natural protein. In one example, the natural protein may be a clearance receptor, e.g., for atrial natriuretic factor, and the inhibitory polypeptide binds to that receptor and thereby raises the level of atrial natriuretic factor in the body. Alternatively, the inhibitory polypeptide may reduce the specific biological activity by reducing the rate of formation of the natural protein by inhibiting activity

of a protease involved in production of that natural protein. For example, the natural protein may be angiotensin II and the inhibitory polypeptide may act upon the converting enzyme which affects production of angiotensin II.

The invention also features supplementation (by an agonist polypeptide) of a naturally occurring inhibitory polypeptide in those patients where increased levels of the inhibitory polypeptide will have benefit in patients without genetic deficiency. Thus, for example the level of naturally occurring α_1-antitrypsin (trypsin as well as elastase inhibitor) can be increased by the method of the invention (by introducing a gene encoding this inhibitor) and thus is useful in ameliorating disease in patients with conditions such as emphysema, chronic obstructive pulmonary disease, and vascular wall weakenings (such as arterial aneurysms and dissections). α_1-antitrypsin can also be used to inhibit other related proteinases for the prevention and/or treatment of inflammatory diseases and arthritis.

Other inhibitory polypeptides of proteinases are useful in treatment and prevention of arthritis and other inflammatory conditions, including rheumatoid arthritis and osteoarthritis. For example, joint destruction in rheumatoid arthritis and other inflammatory joint diseases is reported to be mediated by various proteases (C.E. Brinckerhoff, Arthritis Rheum, 34:1073-5 (1991)). One group of degradative enzymes that is particularly important is the metalloproteinase family (including stromelysin and

collagenase) (J.F. Woessner, Jr., FASEB J, 5:2145-2154 (1991)). The putative role of metalloproteinases in joint destruction is supported by the observation that they are produced by synovium in arthritis and are present in synovial fluid (G.S. Firestein, et al., Arthritis Rheum, 34:1094-105); L.A. Walakovits, et al., Arthritis Rheum, 35:35-42 (1992)). Normally, the destructive action of metalloproteinases is thought to be balanced in vivo by a family of naturally-occurring inhibitors, known as tissue inhibitors of metalloproteinases (TIMP), that reportedly bind to metalloproteinases and prevent them from degrading their substrate (J.F. Woessner, Jr., FASEB J, 5:2142-2154 (1991)). The ratio of metalloproteinase and TIMP gene expression is thought to be a critical determinant of the net tissue destruction in arthritis. Although the TIMP-1 gene is expressed by the rheumatoid arthritis synovium, the amount of protein produced appears to be insufficient to block extracellular matrix destruction. In an attempt to overcome this situation, the present invention features delivery of a TIMP-1 gene to joint tissues in order to increase local TIMP production. Alternatively, genes encoding smaller inhibitory peptides (even as small as 5 - 10 amino acids) could be delivered to the joint to accomplish the same goal. Another way to deliver genes to the joint to increase proteinase inhibitor production is to remove small amounts of synovial fluid or tissue and transfect or infect fibroblasts isolated from these samples with the gene. The cells could be expanded in vitro and then injected into inflamed

joints where they would produce the inhibitory polypeptide. A similar method has been used to deliver a member of the IL-1 family into rabbit joints (G. Bandara, et al., Arthritis Rheum, 35:S193 (1992)).

A second family of proteases important in arthritis, known as serine proteases, could also be targeted by gene therapy. Protease nexin-1 (PN-1) and protease nexin-2 (PN-2) as well as hirudin are examples of proteins that reportedly inhibit these enzymes (B. Bergman, et al., Proc Natl Acad Sci, 83:996-1000 (1986); M. Schapira and P. Patston, Trends Cardiovasc Med, 1:146 - 151 (1991). In addition to directly protecting tissues by inhibiting protein degradation, an indirect benefit will also be observed since these proteins reportedly activate metalloproteinase proenzymes through limited proteolysis. Therefore, serine protease inhibition would simultaneously decrease metalloproteinase-mediated damage. As with metalloproteinase, ex vivo transfection or infection and/or the use of genes encoding smaller peptide inhibitors could also be used.

While the above descriptions provide specific examples for the treatment of hypertension and arthritis, those skilled in the art will recognize that many other conditions can be treated by use of the above methods. For example, Alzheimer's disease, which is characterized by the presence of beta amyloid deposits, may be treated by inhibition of proteases responsible for generating such beta amyloid. Other inflammatory and non-inflammatory diseases

marked by tissue destruction, like inflammatory bowel disease or emphysema can also be treated by inhibiting metalloproteinases or serine proteases. Alternative strategies for using gene therapy to prevent or treat inflammation include the use of a gene encoding peptide inhibitors of cellular adhesion, such as soluble integrins, solubilized forms of integrin ligands, or peptides that directly interfere with integrin-mediated adhesion. Genes that encode anti-inflammatory cytokines, such as IL-10, or that encode polypeptides that inhibit pro-inflammatory cytokines like IL-1 or TNF alpha (G.S. Firestein and N.J. Zvaifler, Arthritis Rheum, 33:768-73 (1990)) will also be beneficial in the treatment of inflammatory diseases.

Proteinases are involved in proliferation or spread of some cancer cells. Such proteinases aid survival of a tumor cell since they promote tissue and vascular invasion. Inhibition of one or more of these tumor proteinases by introduction of a tumor proteinase inhibitory polypeptide (such as TIMP-1 or TIMP-2) using the method of this invention will reduce or prevent tumor growth, spread and metastasis.

When the method of this invention is used in the treatment of vascular disease, other proteinase inhibitory polypeptides are used to constantly inhibit proteinases which elevate blood pressure, e.g., angiotensin converting enzyme, renin or endothelin converting enzyme. Inhibition of the latter proteinase reduces or prevents vasospasm associated with migraine headaches, or rheumatic diseases such as Raynaud's phenomena, scleroderma, and systemic

lupus erythematosus. Such inhibition of vasospasm is also useful in treating ischemic diseases such as angina pectoris, heart attack, stroke, transient ischemic attack, peripheral vascular disease and bowel, skin and renal ischemia. Inhibition of angiotensin converting enzyme is also useful for treatment of angina pectoris, congestive heart failure and the prevention of heart attack and stroke.

Inhibition of this and other proteases by the method of this invention is useful in prevention of vascular remodeling associated with atherosclerosis and coronary reocclusion following percutaneous transluminal angioplasty and coronary artery bypass grafting administered either at the desired site or tissue where needed, or systemically.

Further, inhibition of this and other proteases by the method of this invention is useful in prevention of cardiac remodeling associated with pressure overload, post myocardial infarction hypertrophy, and other forms of heart failure.

The invention includes delivery and expression of one or more genes encoding natural agonist or inhibitory polypeptides of naturally occurring proteins derived from animals, *e.g.*, humans. In addition, the invention includes delivery of one or more genes encoding novel agonist or inhibitory polypeptides, which can be created *de novo* or as derivatives of known agonists or inhibitors to be used in gene delivery, as described below. Genes encoding such agonists or inhibitors can be delivered in any form, including direct

injection of nucleic acid, for example, as DNA or RNA, ex vivo treatment of cells with re-implantation of treated cells, or vector mediated transfer, including such vectors as retroviral, vaccinia, adenovirus, polio, adeno-associated virus, and herpes virus.

This invention can also be used to enhance the antithrombic activity of endothelial cells which can be transfected or infected in vivo. While Dichek and colleagues (Blood, 77, 534-541 (1991)) used a retroviral vector to insert a nucleotide sequence encoding human tissue-type plasminogen activator (TPA), the present invention inserts a sequence encoding an inhibitor or antagonist of prothrombotic mediators. This includes inhibition of thrombin by the in situ formation of peptides such as hirulog (Maranamore, et al., Biochemistry, 29, 7095-7101 (1990)) or proteins such as hirudin (Markwardt, Semin. Thrombosis Hemostatis, 15, 269-282 (1989), or other peptidic inhibitors or antagonists of thrombin (see, for example, Hung, et al., J. Clin. Invest., 89, 440-450 (1992)). These inhibitors may bind at either the proteolytic (active) site or the fibrinogen binding site of the thrombin molecule. Additional inhibitors include PPACK (D-Phe-Pro-ArgCH_2Cl; Kettner and Sahw, Thrombosis Res., 14, 969-973 (1979)). or serine protease inhibitors (Schapira and Patston, Trends Cardiovasc. Med., 1, 146-151 (1991)), as well as activated protein C which appears to downgrade thrombin amplification by factors VIIIa and Va (Gruber, et al., Circulation, 82 578-585 (1990). Inhibitors of factors Xa or peptides/proteins which interfere with the formation, and, hence,

activity of prothrombin, thereby decrease thrombin activity (see for example Eisenberg, Coronary Artery Disease, 3, 1010-1015 (1992)).

An alternative prothrombotic target is the platelet IIb/IIIa receptor. Peptide antagonists containing RGD or KGD sequences have been described (see for example, Nichols, et al., Trends in Pharmacological Sciences, 13, 413-417 (1992)) and claims are made for derivatives thereof or other inhibiting sequences. Thus genes encoding such polypeptides or the natural RGD-containing polypeptides derived from the venom of various vipers such as trigramin (Huang, et al., Biochemistry, 28, 661-666 (1989)), echistatin (Gan, e al., J. Biol. Chem., 263, 19827-832 (1988)), bitstatin (Shebuski, et al., J. Biol. Chem., 264, 21550-556 (1989)), applagin (Chao, et al., Proc. Natl. Acad. Sci. USA., 86, 8050-8054 (1989)) or kistrin (Dennis, et al., Blood, 74, 129a (1989)) can be inserted into endothelial cells to enhance antithrombotic activity. Similarly, polypeptide antagonists of the platelet Ib receptor can be used to inhibit intravascular thrombosis.

Another use of this invention is the transfection or infection of endothelial cells *in vitro* or *ex vivo* (for example, those obtained from human umbilical vessels or from autologous veins) with genes encoding antithrombotic peptides or proteins. These endothelial cells can then be grafted on cardiac valves, vascular stents, or other appropriate prostheses to from an antithrombotic surface

when implanted in vivo, utilizing the antithrombotic approaches outlined above.

The method of this invention and delivery of polypeptide agonist and inhibitors is useful in treating not only humans and animals, but also for treatment of plants.

As can be seen, the invention provides a broad means by which a number of conditions or diseases can be treated. In general, a suitable purified nucleic acid is introduced into a cell (e.g., an endothelial cell, a kidney, blood element, fibroblast, heart, or liver cell) and expression of an agonist or inhibitory polypeptide caused by use of an appropriate gene expression system. The level of activity of the agonist or inhibitory polypeptide can be regulated using standard methodology. For example, a promoter and enhancer region can be selected and provided to be suitable for expression in such a cell, and under certain circumstances to recognize the level of one or more factors relevant to a desired level or expression of the inserted gene. It is preferred in this invention to express compounds which can be continuously produced without feedback control, and therefore inhibitors are especially useful.

Such purified nucleic acid sequences can be introduced not only into patients suffering from those diseases or conditions discussed above, but also into normal patients as a preventative or prophylactic treatment. For example, to the extent that any particular person is known to be susceptible to a specific disease later in life (e.g., susceptibility to cancer growth because of a

family predilection, or because of occurrence of a cancer earlier in life), that person may have a gene encoding an appropriate polypeptide introduced into their body so that the polypeptide is active to prevent occurrence or manifestation of the disease. For example, those patients thought to be susceptible to Alzheimer's disease, may be treated by provision of an inhibitory polypeptide which inhibits a protease which may become elevated in such patients. Occurrence of Alzheimer's disease has been linked to the presence of proteases responsible for generating beta amyloid, thus inhibition of such proteases will prevent early manifestation of Alzheimer's disease. In this instance, these patients are prevented from suffering from this disease by introducing the inhibitory polypeptide-encoding nucleic acid before the patient is at a stage of life when the disease may be manifest, for example, at age 50 or 60.

The phrase "purified nucleic acid sequences" is meant to include any nucleic acid produced by recombinant DNA or RNA technology that is synthesized or isolated from nucleic acid with which it naturally occurs. Preferably it is provided within a vector system, such as a phage, plasmid or virus system, so that it might be introduced into any desired cell. For certain applications, it is particularly preferred in this invention that the vector be formed from a viral system which can be introduced into endothelial cells or fibroblasts to cause expression of a desired polypeptide within such cells or secreted from such cells. Alternatively, endothelial

cells may be infected with a desired gene in a virus or other carrier, and then placed into a patient.

This invention is advantageous over prior methods for inhibiting protein activities in vivo which involved direct administration of an inhibitory polypeptide. The present invention allows controlled and continuous regulation of expression of the inhibitory polypeptide so that sufficient polypeptide is produced as needed by a patient. Since such polypeptides can be expressed constitutively it can be an effective in vivo inhibitor even if the in vivo half-life of the polypeptide is short.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of Preferred Embodiments

The method of this invention, and nucleic acids useful in such methods are generally described above. There follow detailed examples of systems in which such methodology and purified nucleic acid may be used. These examples are not limiting in the invention but are provided purely for illustrative purposes. Those of ordinary skill in the art will recognize that other examples of suitable agonist or inhibitory polypeptides useful in treatment of other diseases and conditions can be used in a method of the invention.

Example 1: Treatment of Hypertension

Hypertension and high blood pressure are regulated by a complex system including an interrelationship between hormones and various proteinases. Below is provided a general outline of various proteins associated with occurrence of hypertension. The method of this invention can be used to interfere with the biological activity of at least one of these protein activities and thus affect occurrence of hypertension.

Renin and Angiotensin

An outline of one system known to be involved in regulation of blood pressure is termed "the renin and angiotensin system" and is described by Cushman and Ondetti, Progress in Medicinal Chemistry, eds. Ellis and West, Elsevier/North-Holland Biomedical Press 42, 1980, hereby incorporated by reference herein. In general, renin and angiotensin converting enzyme are involved in production of angiotensin II, which acts at angiotensin receptors to regulate blood pressure. Renin is an endopeptidase released into the blood from the kidney and causes cleavage of the glycoprotein, angiotensinogen, to produce angiotensin I. This is converted to an octapeptide, angiotensin II, by angiotensin converting enzyme. Angiotensin II is a potent hypertensive and salt-retaining peptide. Angiotensins I and II are also hydrolyzed by peptidases referred to as angiotensinases.

Pharmacological intervention at various steps in the conversion of angiotensinogen to angiotensin II (or the later

produced product angiotensin III) is known to alleviate high blood pressure. For example, antibodies to the various components have been used in treatment to inhibit the various activities, but are depleted from the body and do not necessarily penetrate to the site of action of the target factor. Other inhibitors include various receptor antagonists which act in a competitive fashion, and inhibitors of the various proteases such as both angiotensin converting enzyme and renin.

Cushman and Ondetti, supra, describe the development, mechanism of action, and biological activity of therapeutically useful inhibitors of angiotensin converting enzyme. Effective inhibitors include snake venom peptides and orally active inhibitors which bind specifically at the active site of the enzyme. Other such inhibitors are small polypeptides having between 5 and 9 amino acids, and are known to be efficient inhibitors of angiotensin converting enzyme. Angiotensin converting enzyme is also inhibited to varying degrees by polypeptides that bind to the enzyme in the same manner as substrates or the polypeptide or dipeptide products of the enzyme's action. Thus, a wide variety of peptide inhibitors of this enzyme activity are presently known.

Angiotensin converting enzyme is also able to cause breakdown of bradykinin. Thus, inhibition of the activity of angiotensin converting enzyme is beneficial to a hypertensive patient in two ways since it (a) reduces the level of angiotensin II, and thus reduces blood pressure, and (b) increases the level of

bradykinin which is thought to be responsible for vasodilation, and thus also alleviates high blood pressure.

Other inhibitors of the renin and angiotensin system are described in Goodman and Gillman, The Pharmacological Basis of Therapeutics, 8th Edition, 1991 at chapter 31, page 749 et seq. and in Ondetti, et al., Angiotensin Converting Enzymes Inhibitors: Discovery and Development, page 3 (which discusses teprotide, a 9 amino acid polypeptide inhibitor, and discusses the development of other angiotensin converting enzyme inhibitors), by Henning, et al., Renin Inhibitors, chapter 11, page 483 (which describes use of substrate analogs as inhibitors of renin, as well as inhibitory peptides based on the structure of prorenin), Cushman et al., Enzyme Inhibitors as Drugs , Ed. Sandler, p. 231, Cushman et al., 17 Progress in Medicinal Chemistry 42, 1980, and by Douglas, et al., 116 Endocrinology, 1598, 1985 (which also describes the efficacy of octa- and heptapeptide receptor antagonists of angiotensin II). Such inhibitors are said to be rapidly cleaved by peptidases, and thus have a short duration of action upon intravenous administration.

Two particularly useful examples of polypeptides inhibitory to angiotensin converting enzyme include Pro-Lys-Trp-Ala-Pro and Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro.

Another useful target is a protease involved in production of renin from preprorenin or prorenin. Inhibitors useful in this invention will include polypeptides having an amino acid sequence

similar to the proteolytic target of preprorenin or prorenin. Such an inhibitor is best produced (e.g., by viral transfection) within the juxtaglomerular cell in the kidney so that the peptide inhibitor is within the cells where the target protease is located.

Boger, 20 Annual Reports in Medicinal Chemistry, 257, describes inhibition of renin using antibodies to purified renin, and competitive inhibitors based upon the 13 or 14 amino acid substrate sequence of renin. Boger notes that studies with peptidal competitive inhibitors of renin demonstrate effective blood pressure lowering in renin dependent models when administered as infusions or as bolus injections. Such administration, however, is short acting. Though potent inhibitors of renin have been developed, Boger states that none are reported to have the duration of action or effectiveness characteristic of medicinally useful agents. One example of a polypeptide inhibitory for renin is Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys.

Other useful peptides for decreasing blood pressure in hypertensive animals are described in Cushman and Ondetti, 17 Progress Medical Chemical Chem., eds. Ellis and West, 41, 1980; Burton, et al., 77 Proc. Natl. Sci. USA, 5476, 1980; and Burton, et al., The Design of Substrate Analog Renin Inhibitors in Harubi VB Rich, D.H. editors, Peptides: Structure and Function; Pierce Rockport, IL pages 559-567.

As can be seen from the above discussion, from a review of the cited art, and other art published regarding angiotensin

converting enzyme and renin, potent polypeptide inhibitors of both enzymes exist, and it is recognized that more active polypeptide inhibitors may be generated by analysis of the structure of the target enzyme. For example, Sielecki et al. 243, Science 1346, 1989 describe the X-ray structure of recombinant human renin. This structure can be used as an aid to designing useful polypeptide inhibitors.

Angiotensin converting enzyme is a particularly useful target for inhibition since blockage of the enzyme is possible with minimal ill effect (a cough may occur in some patients). Renin may also be totally blocked, but even reduction of its activity by about 50% within a patient may be sufficient to regulate blood pressure. Surprisingly, the level of renin and angiotensin within most patients having high blood pressure is not significantly different from normal, however, the above-noted peptide inhibitors provide a significant lowering of blood pressure. Thus, even regulating natural occurring levels of these peptides in hypertension has utility in lowering blood pressure. Inhibition of renin in normotensive individuals does not lower blood pressure.

Further, regulating the levels of renin and angiotensin also will have utility in the treatment of heart failure.

Endothelin

Another useful target system for treatment of hypertension and heart failure is the hormone endothelin described generally by Yanagisawa and Masaki, 38 Biochemical Pharmacology, 1877, 1989.

This hormone causes stimulation of atrial natriuretic peptide secretion and inhibits renin release. The hormone itself acts as a vasoconstrictor and has implications in the treatment of hypertension. It is formed from a precursor which is proteolytically cleaved by endothelin converting enzyme. A useful target for treatment of hypertension is endothelin converting enzyme to prevent production of endothelin, and thus reduce hypertension. Since endothelin regulates the level of renin, inhibition of production of endothelin will be particularly useful for treatment of hypertension.

Inhibitors of any target enzyme or hormone or receptor can be identified by methodology equivalent to that used for identifying inhibitors of renin and angiotensin converting enzyme discussed above, and in the art cited above. For example in SCRIP, No. 1592, page 23, February 20, 1991, Immunopharmaceutics, Inc. states that it has synthesized a potential human therapeutic and endothelin receptor antagonist by use of computer aided antibody directed drug design technology. This antagonist is stated to be useful in development of a treatment for hypertension. Thus, polypeptides which act as antagonists to the production or actions of endothelin will be useful in the present invention.

Angiotensin II Receptor

Inhibitors at the angiotensin II receptor are described by Douglas, et al. 116 Endocrinology, 1598 (1985) where angiotensin II analogs containing modifications of the N-terminus and C-terminus

are shown to act as angiotensin II receptor antagonists. One example is Arg-Val-Tyr-Ile-His-Pro-Ile.

Atrial Natriuretic Factor C-receptor

Maack, et al., 238 Science, 675 (1987) describe atrial natriuretic factor C-receptor inhibitors. For example, polypeptide analogs of atrial natriuretic factor are described that lower blood pressure by binding to the atrial natriuretic factor clearance receptor and thus raise endogenous levels of atrial natriuretic factor.

Other Receptors

Other possible targets for treatment of high blood pressure include polypeptides active at a receptor for neuropeptide Y, and other such polypeptides e.g., vasoactive intestinal polypeptide, which are co-released from sympathetic nerves and amplify nerve activity, and thus contribute to high blood pressure. Peptides of the present invention can be administered to block the activity of these target polypeptides on their receptors. Since such receptors are known, such inhibitory polypeptides can be modeled on the target polypeptides themselves to provide inhibition in a competitive manner.

Thus, generally any enzyme, hormone, or polypeptide which acts to elevate blood pressure can be inhibited by the method of the present invention by blocking the activity of that enzyme, hormone or polypeptide by a competitive or non-competitive mechanism using a polypeptide inhibitor produced intracellularly or inhibitor

secreted into the bloodstream or interstitial fluid. Alternatively, an enzyme, hormone or receptor which is useful for reducing blood pressure may be introduced by a method of this invention to act as an agonist to a natural protein. Preferably, such a polypeptide is produced in the endothelial cells, heart cells, or other cells which are in contact with the blood, e.g., the liver, kidney (particularly, for renin) and vascular smooth muscle cells. It is preferable that such cells be replicating cells.

In other systems, agonists or inhibitors of various peptidases or other hormones or enzymes are known as discussed in the Summary of the Invention. The inhibitors may be as short as dipeptides, for example, Phe-Ala described by Llorens, et al., 96 BBRC, 1710 (1980). Kow and Pfaff, 28 Annual Review Pharmacological Toxicology, 163 (1988) describe the neuromodulatory actions of various peptides and notes that vasopressin may be useful in regulation of blood pressure.

The discussion above shows that polypeptide inhibitors and agonists are already known which are suitable for reducing hypertension. Other useful polypeptides can be identified using existing technology to determine those most useful in regulation of hypertension and blood pressure. As described above, such polypeptides may act by inhibiting protease activity, or by binding competitively at a receptor involved in regulation of hypertension (for example, by preventing hormonal binding to that receptor), or

may inhibit activity of enzymes which reduce the level of peptides or hormones which reduce hypertension.

Peptides produced by the present invention are active in vivo. Their production can be maintained at a constant or varied level. Thus, such activity can be maintained for an extended period of time by such continuous production, while exogenous peptide administration would be impractical because of the short half-life of many such peptides.

Methods by which other useful inhibitory polypeptides can be identified are provided below.

Inhibitory Polypeptide Sequence Determination

The references cited above and the accompanying general discussions show that certain polypeptides act as inhibitors or antagonists for a desired target protein. Genes encoding these polypeptides can be used in the method of this invention. In addition, other polypeptides, e.g., with higher in vitro proteinase inhibitory activity, and longer plasma half-lives can be identified by techniques known to aid in polypeptide design and identification. For example, two approaches have shown considerable success in the discovery of potent enzyme or receptor antagonists. One approach uses rational drug design techniques, namely computer modeling and X-ray crystallography. The second approach enables evaluation of a large library of compounds (e.g., 10^7 molecules) for discovery of potent compounds. These two methods are described in detail below.

Computer-Aided Molecular Modeling:

Molecular modeling techniques have proven to be useful in the design of potent antagonists. Several examples now exist where these techniques have been used to optimize a lead compound starting from the X-ray coordinates of the enzyme-inhibitor complex, (e.g., Ealick, et al., 88 Proc. Natl. Acad. Sci. (USA), 11540, 1991, Erickson, et al., 249 Science, 527, 1990, and Ferguson, et al., 34 J. Med. Chem. 2654, 1991. (These publications are hereby incorporated by reference herein.) The methods described are useful in discovery of useful inhibitors of this invention, for example, for discovery of competitive inhibitors. In such a method the structure of the inhibitory polypeptide and target enzyme is studied and modifications of the structure of the inhibitory polypeptide made using rational design criteria to design a optimum polypeptide. Such modified polypeptides (i.e., one having a slightly different amino acid sequence from the lead compound) are then tested in vivo to determine if they are useful inhibitors of the target activity, and thus useful in this invention.

Techniques have been described for the evaluation of protein (and peptide-mimics) binding conformations in the active-site of proteases, (see e.g., Guida et al., 13 Journal of Computational Chemistry, 1992). This information is relevant to the design and analysis of potential high affinity polypeptide antagonists. Peptides in the proper binding conformation can be further evaluated using free energy perturbation techniques to address

differences in binding affinities, e.g., (Kollman, et al., 34 J. Med. Chem., 2654, 1991). These publications are also hereby incorporated by reference herein, and can be followed to provide useful polypeptides in this invention.

Polypeptide Library Screening:

Several methods have been described that enable generation of polypeptide libraries composed of, e.g., 10^7-10^8 randomly-generated hexapeptides, and the subsequent screening of the libraries for polypeptides that have high affinity for the protein target, and/or a specific physical property that is readily screened. Such libraries can be screened by assay of protease inhibitory activity using protocols designed for specific detection of a desired polypeptide inhibitor. Such technology is described generally by Brown, Genetic Engineering News, 1 (January 1992) hereby incorporated by reference herein. The three major methods described in the literature are:

a) screening of a peptide library generated from bacteriophage vectors containing random oligonucleotide sequences: (Parmley and Smith, 73 Gene, 305, 1988, Scott and Smith, 249 Science, 386, 1991, Cwirla, et al., 87 Proc. Nat. Acad. Sci. USA, 6378, 1990);

b) screening of millions of potential compounds using a tea bag approach for the generation of a library of unmodified free peptides: (Houghton, et al., 354 Nature, 84, 1991); and

c) use of a peptide library generated on resin beads or other methods: (Lam, et al. 354 Nature, 82, 1991). This library allows identification of ligand molecules having a high affinity for enzymes, receptors or antibodies.

Peptides in the libraries with high affinity for the target proteins are then identified as outlined in the references (which are hereby incorporated by reference herein).

In Vivo Inhibitor Production

As discussed above, inhibitory polypeptides of this invention are produced in vivo using available techniques or their equivalent. Those of ordinary skill in the art can generate a nucleic acid sequence which encodes the desired inhibitory polypeptide, and provide that nucleic acid sequence adjacent other sequences useful for in vivo expression of the polypeptide. For example, the nucleic acid encoding an inhibitor may be introduced by direct gene transfer or other gene therapy techniques referenced above (and incorporated herein by reference). In one example, the inhibitor is produced as a secreted peptide and thus is provided with pro and prepro sequences and any other modifications necessary for its useful production in vivo. There follows examples of such procedures known in the art. See e.g., Dorner & Kaufman, 185 Meths. Enz. 577, 1990. Of course, the actual methodology used will depend upon the organism being treated, e.g., bacterium or human. Those skilled in the art will recognize the description below as a

preferable guide for in vivo human production. Equivalent techniques can be used in other organisms.

Production of polypeptide inhibitors in the plasma after retroviral infection will require secretion of the polypeptide out of the infected cell. Protein or peptide secretion typically requires the protein to have 16-30 residues (signal sequence) attached to the N-terminus, which enables the protein to be translocated into the endoplasmic reticulum. During this process the signal sequence is cleaved by specific proteases. An example of a signal sequence as described in the literature is Met-Arg-$(Leu)_n$-Pro-Xaa-Ala-Leu-Gly where n is 6-12 and Xaa = Leu, Ala, Leu-Ala or no residue (Kikuchi and Ikehara, 9 Trends in Biotech., 240, 1991). Numerous other sequences have also been noted, e.g., those in preproinsulin, and preproalbumin also can be used in this invention.

Following the signal sequence is the polypeptide itself or a protein that undergoes processing in either the cell or in whole blood to generate the active polypeptide. Numerous examples exist where the polypeptide or protein is initially translated into a prepropeptide (i.e., an N-terminal signal sequence, the active peptide and an N-terminal or C-terminal peptide). Secretion of the active polypeptide involves: (1) translocation into the endoplasmic reticulum and cleavage of the signal peptide and (2) cleavage of the propeptide to the active polypeptide (see In Brain Peptides, (Eds. Krieger, et al.) "Biosynthesis and processing of neuropeptides by Loh and Gainer). The latter process can occur in a variety of ways.

Typically, endocrine cells sort propeptides to a secretory granule which contains the processing enzyme. These enzymes can have high specificities and are localized only in certain cell types (e.g., prorenin is cleaved by a thioprotease in secretory granules found in juxtaglomerular cells). A second secretion pathway (primarily used by non-endocrine cells) involves transfer of the propeptide to membrane-bound vesicles, which mediate constitutive secretion of the propeptide. In this case, cleavage to the active peptide requires extracellular enzymes (either in the blood or on the surfaces of cells that make sufficient contact with propeptides that are secreted into the blood). One example is procollagen. Inhibitory peptides of this invention can be expressed in a system modelled upon any of these types of systems.

Sequences of some cleavage sites are known with the classical site being the dibasic amino acid sequence Lys-Arg (see e.g., Hosaka, et al., 266 J. Biol. Chem., 12,127, 1991). Cleavage may also appear to depend on other parts of the sequence as well the dibasic group. Sequence specificities for some extracellular proteases are known (e.g., for neutral endopeptidase 24.11, and Factor X).

The polypeptide inhibitor can be biosynthesized as a part of a propeptide where the cleaved product would be the peptide inhibitor and a peptide (e.g., N-terminal fragment of insulin, ANF, or a serum protein such as albumin). Another strategy is to create a

peptide containing numerous copies of the peptide inhibitor separated by a peptide sequence containing a cleavable site.

Alternatively, the polypeptide may be produced as a protein with the inhibitory polypeptides positioned within the protein such that inhibition of the desired target is still attainable. Possible sites are at the C-terminus, N-terminus or in a solvent-exposed region of the protein, e.g., within a loop of the protein. An example of such a construction is the binding of hexapeptides expressed on the phage coat protein to receptors or enzymes as described in Parmley and Smith, 73 Gene 305, 1988.

Incorporation of peptides within e.g. a serum protein (e.g., albumin or immunoglobulins) can lead to inhibition of plasma proteases or other enzymes or hormones. A low resolution structure of albumin useful in such a construction has been described.

Suitable systems for expression and delivery of nucleic acid encoding the above inhibitory polypeptides are described by Nabel et al., 249 Science, 1285, 1990, Dick et al., Trends in Genetics, June 1986, Breakfield, EPA 453242, and by Buttrick, et al., 70 Circulation Research, 193, 1992, all hereby incorporated by reference herein. The level of expression of a desired inhibitory polypeptide is chosen to provide a desired level of in vivo inhibition. In one extreme a gene may be expressed constitutively. Alternatively, a promoter or other regulatory region can be chosen to cause gene expression only when a specific level of a chosen

signal is present in the expressing cell. The control and level of expression can be determined by choice of a particular promoter (e.g., with low or high expression level, regulated or not regulated) and by selection of a vector with a copy number of suitable magnitude or by the quantity of vector or transduced cells used.

Example 2. Gene therapy with the TIMP-1 cDNA for adjuvant arthritis in rodents

The following exemplifies a test system for the use of TIMP-1 inhibitor. The model is in rodents but the results can be extrapolated for use in humans. The TIMP-1 cDNA (Nature 315:768 (1985)) is cloned into the polylinker site of pAFVXM. This plasmid can be used to generate infectious vector particles (see below). A dominant selectable marker comprised of an SV40 early promoter driving expression of neomycin phosphotransferase is inserted into the vector to facilitate isolation of infected or transfected cell lines. A promoter, selected from the RSV promoter, SV40 early or late promoter, the CMV immediate early promoter, human beta-bactin promoter, or Moloney murine MLV SL3-3 promoter, is inserted upstream to the TIMP-1 gene in the construct or alternately to insert the TIMP-1 gene into an amphotrophic retroviral vector using the LTR as a promoter and containing the packaging signal.

These plasmids, when placed in a suitable packaging cell, express a retroviral vector construct which contains a packaging

signal. The packaging signal directs packaging of the vector construct into a capsid and envelope along with all further proteins required for viable retroviral particles. The capsid, envelope, and other proteins are preferably produced from one or more plasmids containing suitable genomes placed in the packaging cell. Such genomes may be a proviral construct, which in a simple case may merely have the packaging signal deleted. As a result, only the vector will be packaged. Suitable packaging cell lines and the genome necessary for accomplishing such packaging, are previously described (Mol Cell Bio 6:2895 (1986)). Optionally, further changes may be made into the proviral construct other than simple deletion of the packaging signal in order to reduce the chances of recombination events occurring within the packaging cell line, which may result in production of viral constructs which are not replication defective. The nucleic acid thus produced can be suitably administered to the animal in fashions known to those of skill in the art, e.g., by injection into the requisite site.

To demonstrate the efficacy of this technique, the following experiment is performed. Isolated viral constructs containing either the rodent TIMP-1 cDNA, an inactive TIMP-1 mutant cDNA, or an irrelevant rodent protein cDNA are injected into the rear ankle joints of anesthetized Lewis rats or a susceptible mouse strain. Alternatively, cultured rodent synoviocytes are infected *in vitro* and subsequently injected into the joints. The rats are immunized with complete Freund's adjuvant (0.75 mg of mycobacterium

butyricum in 100 uL of mineral oil) in the base of the tail. If uninhibited, an inflammatory arthritis develops in 8 to 10 days and results in joint destruction and ankylosis over the ensuing weeks (Proc Soc Exp Biol Med 91:95 (1956)). The degree of joint inflammation is quantified by measuring paw volumes and clinical joint scores and paw volumes. Radiographs are obtained and the degree of joint destruction quantified (J Exp Med, 175:1135 (1992)). Injection of the rodent TIMP-1 cDNA offers significant protection against the inflammatory arthritis. In the case of a human drug, the human TIMP-1 gene is used instead.

Other Embodiments

Other embodiments are within the following claims. For example, in certain settings, the invention includes implantation of cells in a region that can be removed, or use of a second gene which will destroy the cell in which it is resident when it is no longer desired, by, for example, the use of a benign compound activated to a toxic form by the conditional lethal gene. Alternatively, administration can be topically in the nose, eyes, urethra, ureter or renal papilla, e.g., for anti-inflammatory treatment or anti-allergy treatment. Nucleic acid which does not integrate into DNA can be used in place of the conditional lethal genes discussed above. In another example, a lethal gene may be activated by shining a light on the patient to turn on a light-activated promoter regulating the lethal gene, e.g., thymidine kinase.

Other diseases or conditions which can be treated by the methods of this invention include those affected by (1) anticoagulants, e.g., a tPA inhibitor or other coagulation factor inhibitors such as protein C or S, (2) complement inhibitors, (3) inhibitors of adhesion molecules such as E-selectin, ICAM or platelet receptor IIb/IIIa, (4) inhibitors of cytokine binding, such as IL-1, (e.g., to treat rheumatoid arthritis) or TNF, for lowering homocysteine levels, or protective apolipoproteins (e.g., for treatment of atherosclerosis or hyperlipidemia), and (6) various antibodies.

CLAIMS

1. A method for treatment of a patient having a normal level of activity of a natural protein or having a condition characterized by the presence of an elevated or reduced level of said natural protein wherein said patient does not have a genetic defect in the structural gene for said natural protein, comprising the step of administering to said patient nucleic acid encoding a polypeptide inhibitor or agonist of the biological activity of said natural protein, wherein said inhibitor or agonist is a polypeptide expressed from said nucleic acid when said nucleic acid is present in said patient, and wherein said inhibitor acts to reduce the specific biological activity of said natural protein in said patient and said agonist acts to increase said specific biological activity.

2. The method of claim 1 wherein said polypeptide inhibitor inhibits a protease, the activity of which causes disease in said patient.

3. The method of claim 1 wherein said polypeptide inhibitor inhibits a protease involved in biosynthesis of a peptide hormone.

4. The method of claim 1 wherein said polypeptide inhibitor inhibits a protease directly involved in a disease process.

5. The method of claim 1 wherein said polypeptide inhibitor inhibits a protease involved in degradation of a beneficial peptide.

6. The method of claim 1 wherein said polypeptide inhibitor antagonizes a peptide binding receptor.

7. The method of claim 1 wherein said patient is an animal.

8. The method of claim 1 wherein said natural protein is selected from a protease, hormone, receptor protein and other enzymes.

9. The method of claim 1 wherein the presence of a normal, reduced or elevated level of said natural protein is a causative element in a disease selected from hypertension, cancer, inflammation, congestive heart failure, cardiac hypertrophy, vascular smooth muscle proliferation, angina pectoris, Alzheimer's disease, emphysema and arthritis.

10. A method of claim 9 wherein said nucleic acid is present in a retroviral vector.

11. A method of claim 10 wherein said polypeptide is selected from TIMP-1 and TIMP-2.

12. A method of claim 10 wherein said polypeptide is selected from PN-1 and PN-2.

13. The method of claim 1 wherein said polypeptide reduces said specific biological activity by direct interaction with said natural protein.

14. The method of claim 1 wherein said polypeptide reduces said specific biological activity by a competitive mechanism.

15. The method of claim 14 wherein said polypeptide binds to a receptor for said natural protein.

16. The method of claim 1 wherein said polypeptide reduces said specific biological activity by reducing the formation of said natural protein by inhibiting activity of a protease involved in production of said natural protein.

17. A method of claim 16 wherein said nucleic acid is present in a retroviral vector.

18. The method of claim 2, 3, 4, 5 or 8 wherein said protease is involved in the control of blood pressure.

19. The method of claim 18 wherein said inhibitor inhibits renin, angiotensin converting enzyme or endothelin converting enzyme.

20. The method of claim 18 wherein said protease is selected from the group consisting of angiotensin converting enzyme, renin, pro-renin protease, neutral endopeptidase 24.11, and endothelin converting enzyme.

21. The method of claim 6 or 8 wherein said receptor is a receptor for atrial natriuretic factor and said polypeptide binds to said receptor and alters the level of atrial natriuretic factor.

22. The method of claim 1 wherein said natural protein is trypsin and said polypeptide is α_1-antitrypsin.

23. The method of claim 22 wherein said α_1-antitrypsin is introduced into a patient suffering from a condition selected from emphysema, pancreatitis, chronic obstructive pulmonary disease, arthritis, and vascular wall weakenings.

24. The method of claim 1 wherein said natural protein is a metalloproteinase and said polypeptide is a tissue inhibitor of metalloproteinase.

25. The method of claim 24 wherein said peptide is produced within synoviocytes.

26. The method of claim 24 wherein said tissue inhibitor of metalloproteinase is selected from TIMP-1 and TIMP-2.

27. The method of claim 24 wherein said tissue inhibitor of metalloproteinase is selected from PN-1 and PN-2.

28. The method of claim 24 wherein said condition is selected from arthritis and other inflammatory conditions.

29. The method of claim 1 wherein said natural protein is a proteinase associated with a tumor cell and wherein said polypeptide is an inhibitor of a said proteinase.

30. The method of claim 1 wherein said condition is vasospasm associated with a migraine headache or rheumatic disease and said polypeptide is an inhibitor of a proteinase associated with said vasospasm or rheumatic disease.

31. The method of claim 30 wherein said rheumatic disease is selected from Raynaud's phenomena, scleroderma and systemic lupus erythematosis.

32. The method of claim 1 wherein said condition is vasospasm associated with an ischemic disease.

31. The method of claim 30 wherein said ischemic disease is selected from angina pectoris, heart attack, stroke, transient ischemic attack, peripheral vascular disease and bowel, skin and renal ischemia.

32. The method of claim 1 wherein said condition is angina pectoris, congestive heart failure, ventricular hypertrophy, heart attack, and stroke and said polypeptide is an inhibitor of angiotensin converting enzyme or renin.

33. The method of claim 1 wherein said condition includes an elevated level of proteinase present after cardiac remodeling associated with pressure overload, post myocardial infarction hypertrophy, and other forms of heart failure and said polypeptide is an inhibitor of said proteinase.

34. The method of claim 1 wherein said condition includes an elevated level of proteinase present after vascular remodeling and said polypeptide is an inhibitor of said proteinase.

35. The method of claim 34 wherein said vascular remodeling is selected from percutaneous transluminal angioplasty,

atherectomy, and coronary artery bypass grafting, and said vascular remodeling is associated with atherosclerosis and coronary occlusion.

36. A method for treatment of a patient suffering from hypertension comprising the step of administering to said patient nucleic acid encoding an inhibitor of the biological activity of a protein associated with existence of said hypertension, wherein said inhibitor is a polypeptide expressed from said nucleic acid when said nucleic acid is present in said patient and acts to reduce the specific biological activity of said natural protein in said patient.

37. The method of claims 1 or 36 wherein said nucleic acid is RNA.

38. The method of claim 36 wherein said inhibitor is an inhibitor of renin, angiotensin converting enzyme renin or endothelin converting enzyme.

39. The method of claim 36 wherein said inhibitor acts at a receptor involved in regulation of blood pressure.

40. The method of claim 39 wherein said receptor is a receptor for neuropeptide Y or vasoactive intestinal polypeptide,

and wherein said inhibitor is a competitive inhibitor of a peptide which naturally binds to said receptor.

41. The method of claim 36 wherein said inhibitor competitively inhibits the activity of an enzyme, hormone, or protease.

42. The method of claim 36 wherein said inhibitor is produced intracellularly, or is secreted.

43. The method of claim 42 wherein said peptide is produced within an endothelial cell.

44. The method of claim 42 wherein said inhibitor is produced within a liver, kidney, or heart cell or fibroblast.

45. The method of claim 39 wherein said natural protein is a receptor for atrial natriuretic factor, and said inhibitor is a polypeptide able to bind at the C- receptor for atrial natriuretic factor and thereby elevate the level of atrial natriuretic factor within said patient and lower blood pressure.

46. A method for treatment of a patient suffering from arthritis comprising the step of administering to said patient nucleic acid encoding an inhibitor of the biological activity of a

protein associated with existence of said arthritis, wherein said inhibitor is a polypeptide expressed from said nucleic acid when said nucleic acid is present in said patient and acts to reduce the specific biological activity of said natural protein in said patient.

47. The method of claims 1 or 46 wherein said nucleic acid is RNA.

48. Purified nucleic acid able to cause expression <u>in vivo</u> of a polypeptide which acts to reduce the specific biological activity of a natural protein present in a patient, said nucleic acid including one or more exons encoding said polypeptide, wherein said exons may be separated by one or more introns or may be adjacent to each other, a signal sequence able to cause secretion of said polypeptide from a cell including said purified nucleic acid, and control sequences able to regulate the level of expression of said polypeptide within said cell.

49. The purified nucleic acid of claim 48 wherein said polypeptide is an inhibitor of a protease, a hormone or an enzyme, or is able to bind at a cell receptor.

50. The purified nucleic acid of claim 49 wherein said polypeptide is an inhibitor of angiotensin converting enzyme, endothelin converting enzyme, or renin.

51. The method of claim 1 wherein said natural protein is a neutral metalloproteinase, stromelysin or collagenase and wherein said inhibitor is an inhibitor of said stromelysin or collagenase or of an enzyme involved in the conversion of prostromelysin to stromelysin.

52. The method of claim 51 wherein said inhibitor is selected from TIMP-1 or TIMP-2.

53. The method of claim 51 wherein said inhibitor is selected from PN-1 or PN-2.

54. The method of claim 1 wherein said natural protein affects the level of preprorenin or prorenin and said inhibitor polypeptide reduces the level of renin in said patient.

55. The method of claim 54 wherein said natural protein is prorenin protease.

56. The method of claim 54, wherein said prorenin protease inhibitor is produced within juxtaglomerular cells.

57. The method of claim 1 wherein said natural protein is selected from the group consisting of adhesion molecules, cytokines, and anticoagulants.

58. The method of claim 1 wherein said natural protein is a protective apolipoprotein, and said patient suffers from atherosclerosis or hyperlipidemia.

59. The method of claim 1, 36 or 46 wherein said nucleic acid is present in a retroviral vector.

60. The method of claim 1 wherein said protein is thrombin.

61. The method of claim 61 wherein said polypeptide is a antagonist said receptor being selected from platelet IIb/IIIa receptor and platelet 1b receptor.

62. A method of claim 24, 25, 26, or 27 wherein said nucleic acid is RNA.

63. A method of claim 24, 25, 26, or 27 wherein said nucleic acid is present in a retroviral vector.

64. A method according to claim 1 wherein the naturally occurring protein is fibrinogen and said polypeptide is an antagonist capable of binding to the platelet IIb/IIIa receptor or to the platelet 1b receptor.

65. The method of claim 1 wherein said natural protein is involved in proliferation or spread of cancer cells.

66. The method of claim 65 wherein said inhibitor is selected from TIMP-1, TIMP-2, PN-1 and PN-2.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02819

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :A61K 48/00, C07H 21/00; C12N 15/00
US CL :514/44, 536/27, 435/320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/44, 536/27, 435/320.1, 172.3; 424/9, 93B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Blood, Volume 76, No. 2, Issued 15 July 1990, Miller, "Progress toward human gene therapy", pages 271-278, see the entire document.	1-66
Y	Science, Volume 249, Issued 14 September 1990, Nabel et al., "Site-specific gene expression in vivo by direct gene transfer into the arterial wall", pages 1285-1288, see the entire document.	1-66
Y	US,A, 4,497,796 (Salser et al.) 05 February 1985, see the entire document.	1-66

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemistry, Volume 19, No. 22, Issued 1971, Ondetti et al., "Angiotensin-converting enzyme inhibitors from the venom of Bothrops jararaca. Isolation, elucidation of structure, and synthesis", pages 4033-4039, see the entire document.	1-66
Y	Agric. Biol. Chem., Volume 53, No. 4, Issued 1989, Maruyama et al., "Angiotensin I-converting enzyme inhibitory activities of synthetic peptides related to the tandem repeated sequences of a maize endosperm protein", pages 1077-1081, see the entire document.	1-66
Y	WO,A, 89/11657 (Sheppard et al.) 30 November 1989, see the entire document.	1-66
Y	WO,A, 90/03181 (Hanley et al.) 05 April 1990, see the entire document.	1-66
Y	Journal of Medicinal Chemistry, Volume 31, No. 9, Issued 1988, Hui et al., "Design of rat renin inhibitory peptides", pages 1679-1686, see the entire document.	1-66
Y	Proceedings of the National Academy of Sciences, USA, Volume 83, Issued February 1986, Bergman et al., "Inhibition of tumor-cell-mediated extracellular matrix destruction by a fibroblast proteinase inhibitor, protease nexin I, pages 996-1000, see the entire document.	1-66
Y	Science, Volume 238, Issued 30 October 1987, Maack et al., "Physiological role of silent receptors of atrial natriuretic factor", pages 675-678, see the entire document.	1-66
Y	The FASEB Journal, Volume 5, No. 8, Issued May 1991, Woessner, "Matrix metalloproteinases and their inhibitors in connective tissue remodeling", pages 2145-2154, see the entire document.	1-66

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG (files 154, 55, 312, 311), US Automated Patent System (file USPAT, 1975-1993).
Search terms: gene therapy, peptide, agonist, hypertension, angiotensin, renin, express, TIMP, PN, nexin, mas, antagonist, inventors' names.